EXPERIMENTS WITH CHRYSOMELID BEETLES.[1]

ROBERT W. HEGNER.

Results obtained by the writer within the last five years from experiments upon *Calligrapha bigsbyana* and other chrysomelid beetles have shown that the eggs, larvæ, pupæ and adults of these insects are admirably suited for the study of many of the external and internal factors of development. The eggs may be definitely oriented with regard to the future position of the embryo quite easily; they continue to develop when subjected to extremely violent mechanical conditions; and various parts may be removed without retarding their development (Hegner, 1908*a*, 1908*b*, 1909*a*, 1909*b*). The larvæ are easily reared in the laboratory in an environment similar to that which they encounter in nature; they usually thrive well under experimental conditions. The same things may be said of the pupæ and adults.

It is the writer's intention to present in this paper, and those that are to follow, the data and conclusions derived from experiments dealing with the growth and external and internal factors which influence the development of the various stages in the life history of certain chrysomelid beetles. Thus far the willow beetles, *Calligrapha bigsbyana* and *C. multipunctata*, have received the largest share of attention, but other species have also been employed.

I. The Normal Rate of Growth of *Calligrapha Bigsbyana*.

1. *The Weight of Developing Eggs.*

Method and Data.—Two series of weighings were made to determine the loss of weight of developing eggs of *C. bigsbyana*. Table I. shows the results for twelve eggs laid by four different beetles at practically the same time. Two batches of two eggs each, one of three, and one of five were taken at 1 P.M. on June 29, placed in a watch glass, and covered by another watch glass. The loss in weight is quite striking. The pigmentation of the

[1]Contributions from the Zoölogical Laboratory of the University of Michigan, No. 128.

embryos became visible through the chorion on the sixth day (July 5). However, the eggs did not hatch, as is usually the case, on the fifth or sixth day (Hegner, 1908*a*), nor on the seventh or eighth day. Loss of water no doubt prevented the larvæ from breaking through the chorion.

TABLE I.

WEIGHINGS MADE OF 12 EGGS OF *Calligrapha bigsbyana* LAID BY 4 BEETLES AT 1 P.M., ON JUNE 29, 1909. AVERAGE TEMPERATURE, 23°C.

Date.	Total Weight in mgs.	Average Weight per Egg in mgs.	Total Loss in mgs.	Average Loss per Egg in mgs.
June 29	9	.75		
June 30	8	.6666	1	.0834
July 1	7.2	.6	.8	.0666
July 2	6.7	.5583	.5	.0417
July 3	6.1	.5083	.6	.05
July 4	5.8	.4833	.3	.025
July 5	5.1	.425	.7	.0583
July 6	4.4	.3666	.7	.0583
July 7	3.8	.3166	.6	.05

TABLE II.

WEIGHINGS MADE OF 22 EGGS OF *Calligrapha bigsbyana* LAID BY 5 BEETLES AT 4 P.M. ON JULY 7, 1909. AVERAGE TEMPERATURE, 25° C.

Date.	Total Weight in mgs.	Average Weight per Egg in mgs.	Total Loss in mgs.	Average Loss per Egg in mgs.
July 7	15.2	.6909		
July 8	15	.6818	.2	.0091
July 9	14.8	.6727	.2	.0091
July 10	14.1	.6409	.7[1]	.0318
July 11[2]	13.5	.6136	.6	.0273

It has been my custom to keep a small piece of filter paper, moistened with distilled water, in the watch glass with the eggs to prevent desiccation. Too much moisture frequently enables a fungus to establish a growth upon the chorion, sometimes checking the hatching of the eggs, whereas too little moisture may also prevent hatching. In nature the eggs are laid on the under surface of leaves where they are kept sufficiently moist by the condensation of water vapor at night.

[1]The moistened filter paper was removed on July 9th and returned on July 10. This accounts partly for the comparatively great loss in weight during this interval.

[2]Twenty of the eggs hatched on July 12. The chorions from which the larvæ escaped weighed 1.176 mg., or .0588 mg. per chorion.

The eggs whose egg weights are recorded in the second series (Table II.) were placed in a covered watch glass along with a piece of filter paper which was moistened with a drop or two of water every day. The loss in weight of these twenty-two eggs during embryonic development was not nearly so great as was that of the first series (Table I.), and doubtless represents more closely the state of affairs under normal conditions. Twenty of the twenty-two eggs hatched on the fifth day, the usual time for eggs of this beetle.

Discussion and Conclusions.—The belief has been held for many years that eggs diminish in weight during the early embryonic stages, and before extraneous food is consumed. That this belief is well founded has been proved by careful experiments with the eggs of several species of animals.

Pott and Preyer (1882) have shown that the hen's egg loses weight during incubation. The amount of oxygen absorbed by the eggs equaled the amount of CO_2 excreted. This excretion, produced in the physiological processes taking place during incubation, does not, at least in this case, account for the loss in weight, as is usually supposed, since the decrease is equalized by the absorption of oxygen. The conclusion was reached that a gradual evaporation of the albumen caused the loss in weight.

When hens' eggs are incubated in desiccators the rate of development is accelerated during the first three days, but later is retarded, and many of the embryos become abnormal or die (Féré, 1894).

Eggs that develop in water have also been used to determine the loss in weight of developing eggs during development (Ritter and Bailey, 1908). Bailey used for his experiments the eggs of the California mud-fish, *Fundulus parvipinnis*. Starting ten days after ensemination, 93 eggs were weighed at intervals of about 20 hours, covering a period of 9 days. Of the 36 weighings made, only 10 showed a gain, and this was accounted for by the presence of dirt upon the eggs. Bailey believes that the "loss in weight must have been due to carbon dioxide (CO_2) and organic salts representing the albuminoid loss, which had passed out through the egg-membrane and been washed away in the sea-water."

A loss of energy also takes place during segmentation, and, in

the case of the sea-urchin egg, has actually been measured, though not enough experiments were performed to make the resultant figures of much value (Spaulding, 1907).

The eggs of chrysomelid beetles differ in several respects from any thus far used for weight experiments. In the first place they are covered by a chitinous chorion which is comparatively impervious to fluids, and is especially well adapted to withstand desiccation. The method of cleavage, *i. e.*, superficial, differs from that of the eggs heretofore examined.

The results of the two series of weighings recorded in Tables I. and II. prove conclusively that there is a loss in weight, and that this loss is largely due to evaporation. A comparison of the data in Tables I. and II. shows this quite clearly, since the eggs weighed in Table I. were allowed to develop without the addition of moisture, and consequently decreased in weight more rapidly.

2. *The Rate of Growth of Larvæ, Pupæ and Adults.*

Method and Data.—The twenty larvæ that hatched from the eggs used in determining the loss in weight of developing eggs (see Table II.) were weighed daily until they pupated; the pupæ were then weighed daily, and finally the adults. These weighings extended over the period from July 12 to August 14, 1908. Because of the daily disturbances made necessary by the weighings, many of the larvæ died. This mortality was greatest during the first four days; however, under normal conditions, many of the larvæ die during this early stage.

The data obtained have been arranged chronologically in Table III. Fig. 1 gives the curve showing the daily increase in weight and Fig. 2 gives the curve showing the daily percentage increments in weight.

Discussion and Conclusions.—The problem of growth is one of great interest to zoölogists, and its study has been given added impetus by the work of Minot (1891, 1907). This investigator considered growth not as in increase in size or volume, but as an increase in mass or weight. The rate of growth was measured by him by taking the increase in weight during a definite period and expressing it as a percentage of the weight at the beginning of that period. Any change in weight can thus be shown by successive percentages for equal periods of time.

TABLE III.

THE RATE OF GROWTH OF LARVÆ, PUPÆ AND ADULTS OF *Calligrapha bigsbyana.*

Date 1909.	Age in Days.	No. of Larvæ.	No. of Pupæ.	No. of Adults.	Total Weight in mg.	Average Weight in mg.	Daily Increase in Weight in mg.	Per Cent. Daily Increase.
July 12		20			11.3	.565		
July 13	1	20			12.	.6	.035	6.2
July 14[1]	2	20			12.6	.63	.03	5.
July 15[2]	3	17			19.	1.118	.488	77.4
July 16[3]	4	9			15.6	1.733	.615	55.
July 17	5	9			17	1.889	.156	9.
July 18	6	9			34.2	3.8	1.911	101.1
July 19[4]	7	9			39.4	4.377	.577	15.2
July 20[5]	8	8			44.6	5.575	1.198	27.3
July 21	9	8			58	7.25	1.575	28.2
July 22	10	8			107	12.375	5.125	70.7
July 23[6]	11	8			134	16.75	4.375	35.3
July 24	12	8			140.5	17.582	.832	4.9
July 25	13	8			193.5	24.187	6.605	37.5
July 26	14	8			287	35.875	11.688	48.3
July 27[7]	15	7			294.8	42.114	6.239	17.4
July 28[8]	16	7			284	40.57	— 1.544	— 3.6
July 29[9]	17	6			249	41.5	.93	2.2
July 30	18	6			235	38.917	— 2.583	— 6.2
July 31	19	6			257	42.833	3.916	10.
August 1	20	6			257	42.833	0	0
August 2	21	6			259	43.166	.333	.77
August 3	22	6			250	41.666	— 1.5	— 3.4
August 4	23	6			236	39.33	— 2.333	— 5.6
August 5	24	6			239	39.833	.5	1.2
August 6	25	4	2		242	40.33	.5	1.2
August 7	26	3	3		238	39.66	— .67	— 1.6
August 8	27	3	3		235	39.166	— .5	— 1.2
August 9	28	2	4		234	39.	— .166	— .42
August 10	29	2	4		235	39.166	.166	.42
August 11	30	2	4		234	39.	— .166	— .42
August 12	31	1	2	3	231	38.5	— .5	— 1.25
August 13[10]	32		2	3	194	38.8	.3	.77
August 14	33		2	3	190	38.	— .8	— 2.06

[1]Larvæ began to feed.

[2]Three larvæ died.

[3]Moulting began on the fourth day; eight larvæ died.

[4]Second moult in progress.

[5]One larva died.

[6]Third moult in progress.

[7]One larva died.

[8]Feeding practically stopped and larvæ prepared for pupation.

[9]One larva died.

[10]One larva died.

Minot's results from weighings made of guinea-pigs show that the growth rate increases almost immediately after birth, the decline being very rapid at first, but less rapid as the age of the

animals increases. That there is a corresponding prenatal decline in the rate of growth was shown by means of rabbit embryos. Curves representing the change in the rate of growth with age

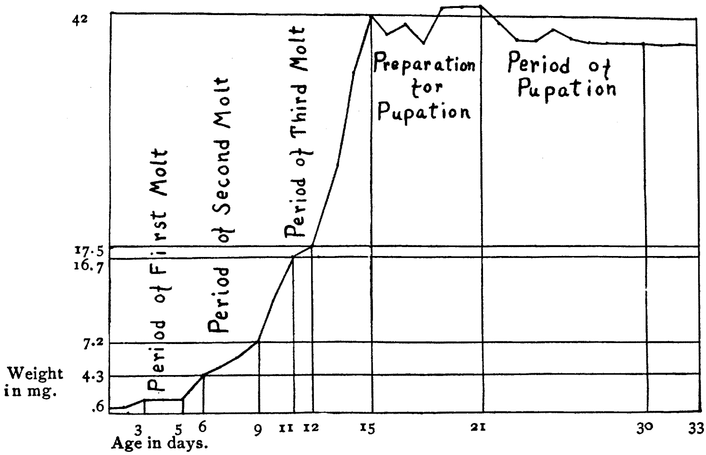

FIG. 1.

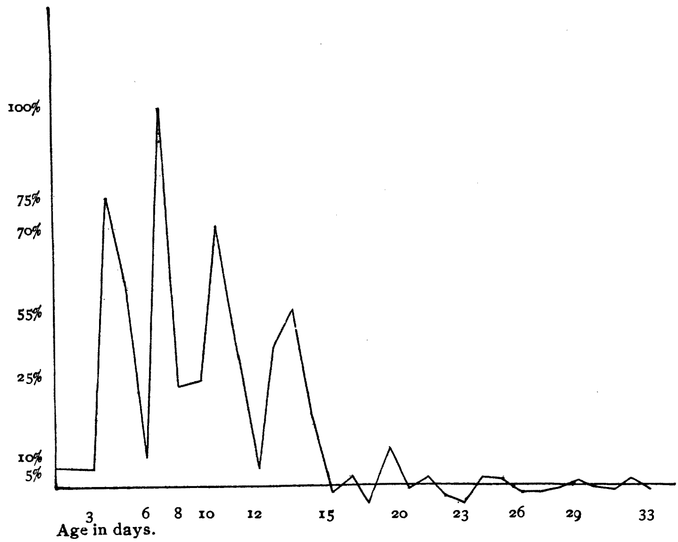

FIG. 2.

have been constructed for the embryos and young of many animals, and almost without exception the growth-rate declines as development proceeds.

Davenport (1897) has shown for the tadpoles of *Rana*, *Bufo* and *Amblystoma*, that, during the first two weeks of larval life, growth is largely due to the absorption of water, which increased from 56 to 96 per cent. During later development, however, the storing up of formed substances is mainly accountable for their growth. The curve of the growth-rate for tadpoles does not agree with the general rule; it rises first, then declines, and finally rises again. This result is probably due to the absorption of water.

TABLE IV.

THE RATE OF GROWTH OF LARVÆ OF *Telea polyphemus* (TROUVELOT, 1867).

Age in Days.	Weight in Grains.	Increase in Weight in Grains.	Per Cent. Increase.
Just hatched	.05		
10	.5	.45	900
20	3	2.5	500
30	31	28	933
40	90	59	190
56	207	117	130

Trouvelot (1867) has given a few weighings of the larvæ of the moth, *Telea polyphemus*. These have been arranged in Table IV. so as to show the actual increase in weight, and also the percentage increments for ten day intervals. The decline in the rate of growth is not regular, probably because of the meager data, but it is no doubt similar to that exhibited by the guinea-pig and other animals.

Fig. 1 shows the weight of developing beetles of the species *C. bigsbyana* from the time of hatching to the emergence of the adults, a period of 33 days. The following data will make clear certain irregularities in the curve. The larvæ usually devour a part or all of their cast-off egg-shells soon after hatching, but do not begin to feed actively until the second day; this accounts for the very slight increase in weight during the first two days. An actual decrease in weight would be expected at the moulting periods, when food-taking ceases and the chitinous covering is shed, but all larvæ do not moult at the same time (see Table V.). and instead of a decrease in the average weight, there is a slight increase. This is shown in all of the moults. The period of most rapid increase is that between the fifth and the fifteenth

TABLE V.

THE WEIGHT OF INDIVIDUAL LARVÆ OF *Calligrapha bigsbyana* WHEN 7 DAYS OLD; JUST AFTER THE FIRST MOULT.

Date of Moulting.	Weight in mgs.
July 16	5.8
July 16	5.4
July 16	5.4
July 17	4.8
July 17	4.6
July 17	4.
July 18	2.6
July 18	2.
July 19	.9

days. From the latter time onward the larvæ gradually cease feeding and lie on their backs in the earth provided for them. During this preparation for pupation, and during the period of pupation, there is a steady decline in weight until the adults emerge.

Fig. 2 shows the daily percentage increments in the weight of the developing beetles. The remarks made in explanation of Fig. 1 also explain the irregularities in this curve. The percentage increments decline very rapidly during the moulting periods. If all of the larvæ moulted at the same time, the rate would be negative.

The data obtained from these weighings confirm what Minot (1891, 1907) has found to be true of guinea-pigs, *i. e.*, the rate of growth declines rapidly during the early stages of development and more slowly during the later stages. Jenkinson (1909) has obtained similar results for many other animals by using the data already available in literature.

II. THE EFFECTS OF LIGHT UPON THE DEVELOPMENT OF *Calligrapha Bigsbyana*.

1. *The Influence of Darkness.*

Method and Data.—Experiment C.B. 42. Eight eggs of *C. bigsbyana* were laid at 12 M., June 10. Four were allowed to develop in an ordinary stender dish (7 cm. in diameter), and the other four were placed in a similar receptacle which had been covered externally with a coat of opaque paint. The same amount of moisture was supplied to each dish, and the temperature did not vary a degree.

Two eggs in the light and all of those in darkness hatched on June 19; the two remaining in the light hatched on June 20. On June 23 two of the larvæ in the darkness moulted. Three of those in the light died on June 23; the other moulted on June 24, as did the two remaining in the dark. All of the larvæ were accidentally destroyed on June 25.

Experiment C.B. 70. Four batches of eggs were laid by four different beetles at approximtaely 10:30 A.M. June 26. One half of each batch were allowed to develop in the light several feet from a window; the other half were placed in darkness as in experiment C.B. 42. The conditions of moisture and temperature were similar in the two dishes. The data have been arranged in Table VI.

TABLE VI.

DATA RECORDED IN EXPERIMENT C.B 70, SHOWING THE RATE OF DEVELOPMENT OF EGGS, LARVÆ AND PUPÆ OF *Calligrapha bigsbyana* IN WHITE LIGHT AND IN DARKNESS.

Date 1909.	White Light,	Darkness.
June 26	15 fresh eggs from 4 batches	16 fresh eggs from 4 batches
July 1—8 A.M.	5 hatched	5 hatched
July 1—1 P.M.	2 hatched	2 hatched
July 2	7 larvæ	2 hatched
July 3	7 larvæ	9 larvæ
July 4	7 larvæ	8 larvæ alive 1 larva dead
July 5	1 moulted	
July 6	4 moulted	6 moulted
July 7	1 second moult 1 still in 1st instar	1 dead
July 8	2 second moult 1 still in 1st instar	1 second moult 1 dead
July 18	5 ready to pupate	4 ready to pupate
July 21	2 pupæ	1 pupa
July 22	4 pupæ	4 pupæ
July 23	5 pupæ	5 pupæ
July 26	5 pupæ	6 pupæ
July 28	2 adults	1 adult
July 29	3 adults	3 adults
July 30	5 adults	5 adults
August 1	5 adults 2 larvæ did not pupate	5 adults 1 adult did not emerge

Discussion and Conclusions.—The eggs of *C. bigsbyana* are attached to the under surface of the leaves of the food plant of the larvæ, *Salix longifolia*, and are thus never exposed to the direct rays of the sun except for exceedingly brief intervals when the leaves twist in the wind. They develop therefore in light of

moderate intensity. Eggs that develop within an opaque mother, or that possess an opaque envelope, pass through their embryonic stages in darkness; but there can be no doubt that the chorion of the beetle's egg allows the light to penetrate, since, as I shall show in a later paper, sunlight has a decided influence upon embryonic development.

In certain cases experiments have seemed to prove that darkness delays the growth of the eggs or larvæ, *e. g.*, Yung (1878) recorded not only a retardation in the development of frog larvæ, but also a high death rate. The same investigator noted a slight retardation in the development of the eggs of the snail, *Lymnæa stagnalis*, when placed in the dark.

Vernon (1895), on the other hand, found that echinoderm larvæ suffer very little, if any, change from the normal when reared in absolute darkness. Loeb (1896) also has brought forth evidence proving that darkness does not retard the embryonic development of the fish *Fundulus*, but does effect a decrease in the number of pigment cells on the yolk-sac.

In other cases, darkness does not hinder the growth of the embryo or larva, but fails to stimulate the hatching process. Przibram (1906) found that the larvæ of the praying mantis, *Sphodromantis bioculata*, are retarded if the cocoon is placed in the dark.

In discussing experiments C.B. 42 and C.B. 70, the normal rate of development and its variations must be noted. Records of over 2,000 eggs of *C. bigsbyana* and the closely allied species *C. multipunctata* give 5 days and 16 hours as the average hatching period (Hegner, 1908*a*). This period varies according to conditions of moisture, temperature and probably other external factors, from 4 to 7 days. Records were also made of over 1,000 larvæ. The average larval life is 20 days; but, as in the case of the hatching time, this period may be shortened to 17 days or extended over 24 days by differences in external conditions. The average pupal period is 12 days, though adults frequently emerge in a shorter time, and a few do not escape until 13 or 14 days have elapsed. These variations in the duration of the different stages may occur in eggs, larvæ or pupæ from different batches of eggs or from the same batch.

The data from experiments C.B. 42 and C.B. 70 indicate that darkness has no retarding nor accelerating influence upon the embryonic development, upon the rate of larval growth, or upon the period of pupation.

One other conclusion that may be arrived at from these experiments is that darkness has no effect upon the coloration of the eggs, larvæ, pupæ or adults of the species studied. Frequent examinations were made during the growth of the beetles reared in the dark, but no variations from the normal were discovered that could be attributed to the absence of light. This confirms Przibram's (1906) results for the praying mantis, the entire post-embryonic development of which was carried out in the dark without producing any effect upon the coloring.

2. *The Influence of Colored Lights.*

Method and Data.—Experiment C.B. 64. This experiment is the only one attempted with a view to testing the effects of colored lights upon the embryonic development of beetles' eggs; but it indicates that color has no very striking influence upon the rate of development.

Several eggs from a single batch of 15, which were laid at 10:30 A.M. on June 24, were placed in each of six cylindrical tubes. These tubes were then closed with rubber corks through each of which were inserted a thermometer and a tube for ventilation. These cylindrical tubes were then immersed in different colored liquids prepared according to Yung (1878). The colors used were red, blue, yellow, green and violet, and a tube was kept in pure water as a control. The temperature in the different tubes was practically identical. The eggs in the white, yellow, green and red lights hatched on June 29; those in the violet and blue were ready to hatch on the same day, but were prevented by fungus growths.

Discussion and Conclusions.—Many experiments have been performed with eggs of a number of species of animals to determine the influence of colored lights upon their development. Yung (1878) used freshly laid eggs of the frog, *Rana esculenta* and *R. temporaria*. At the end of two months all of the tadpoles in the green light were dead, those in the white and yellow lights

were greater in number, those in the red light were retarded and finally died, and those in the violet light were larger, but less advanced and had greater powers of resistance.

These results have not been confirmed for the frog and other animals by later investigators. For example, Vernon (1895) found that the larvæ of echinoderms, in some cases, were not killed by the green light, and that yellow light caused greater injury than red. Driesch (1892), moreover, claims that the eggs of *Rana*, *Echinus* and *Planorbis* are not influenced by any of these colors.

In experiments on the praying mantis, Przibram (1906) found that the influence of green, red and yellow glasses was unfavorable, though this may have been due to differences in the temperature, which was not controlled.

My experiment with the eggs of *C. bigsbyana* confirms for the eggs of this beetle the results obtained by Driesch for eggs of *Rana*, *Echinus* and *Planorbis*.

Zoölogical Laboratory,
University of Michigan,
April 15, 1910.

LITERATURE.

Davenport, C. B.
'97 The Rôle of Water in Growth. Proc. Boston Soc. Nat. Hist., Vol. 28.

Driesch, H.
'92 Entwicklungsmechanische Studien, 2. Zeit. f. wiss. Zool., Bd. 53.

Féré, C.
'94 Note sur l'influence de la deshydratation sur le developpement de l'embryon de poulet. C. R. Soc. Biol., Tome 10.

Hegner, R. W.
'08*a* Observations on the Breeding Habits of Three Chrysomelid Beetles, Calligrapha bigsbyana, C. multipunctata and C. lunata. Psyche, Vol. 15.
'08*b* The Effects of Removing the Germ-Cell Determinants from the Eggs of Some Chrysomelid Beetles. Biol. Bull., Vol. 16.
'09*a* The Effects of Centrifugal Force upon the Eggs of Some Chrysomelid Beetles. Journ. Exp. Zool., Vol. 6.
'09*b* The Origin and Early History of the Germ-Cells in Some Chrysomelid Beetles. Journ. Morph., Vol. 20.

Jenkinson, J. W.
'09 Experimental Embryology.

Loeb, J.
'96 Ueber den Einfluss des Lichtes auf die Organbildung bei Thieren. Pflüger's Arch., Bd. 63.

Minot, C. S.

'91 Senescence and Rejuvenation. Journ. Phys., Vol. 12.

'07 The Problem of Age, Growth, and Death. Pop. Sc. Month., Vol. 70–71.

Pott, R., und W. Preyer.

'82 Ueber den Gaswechsel und die chemischen Veränderungen des Hühnereies während der Bebrütung. Pflüger's Arch., Bd. 27.

Przibram, H.

'06 Aufzucht, Farbwechsel und Regeneration einer ägyptischen Gottesanbeterin (Sphodromantis bioculata Burm). Arch. Entwm., Bd. 22.

Ritter, W. E., and S. E. Bailey.

'08 On the Weight of Developing Eggs. Univ. of Cal. Pub., Vol. 6.

Spaulding, E. G.

'07 The Energy of Segmentation. Journ. Exp. Zool., Vol. 4.

Trouvelot, L.

'67 The American Silkworm. Am. Nat., Vol. 1.

Vernon, H. M.

'95 The Effect of Environment on the Development of Echinoderm Larvæ: an Experimental Inquiry into the Causes of Variation. Phil. Trans. Roy. Soc., Vol. 186.

Yung, E.

'78 De l'influence des milieux physiques sur les êtres vivants. Arch. Zoöl. Exp. et Gèn., Tome 7.

'81 Des l'influence des lumières colorées sur le développement des animaux. Mitt. Zoöl. Stat. Neapel., Bd. 2.